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Cytology of ten species in *Anemone*, one in *Anemoclema* and six in *Clematis* (Trib. Anemoneae, Ranunculaceae) from China

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Abstract The somatic chromosome number and detailed chromosome morphology have been studied in ten species of *Anemone*, one species of *Anemoclema*, and six species of *Clematis*, all from China, namely *Anemone davidii* Franch. ($2n = 4x = 32$), *A. stolonifera* Maxim. ($2n = 2x = 16$), *A. flaccida* Fr. Schmidt ($2n = 2x = 14$), *A. rivularis* Buch.-Ham. ($2n = 2x = 16$), *A. begoniifolia* Lévl. et Vant. ($2n = 2x = 16$), *A. hupehensis* Lem. f. *alba* W. T. Wang ($2n = 2x = 16$), *A. tomentosa* (Maxim.) Péi ($2n = 2x = 16$), *A. rupestris* Hook. f. et Thoms. ($2n = 2x = 14$), *A. trullifolia* var. *colestina* (Franch.) Finet et Gagnep. ($2n = 2x = 14$), *A. trullifolia* var. *holophylla* Diels ($2n = 2x = 14$), *A. demissa* Hook. f. et Thoms. ($2n = 2x = 14$), *Anemoclema glaucifolium* (Franch.) W. T. Wang ($2n = 2x = 16$), *Clematis kockiana* Schneid. ($2n = 2x = 16$), *C. rehderiana* Craib. ($2n = 2x = 16$), *C. ranunculoides* Franch. ($2n = 2x = 16$), *C. puberula* var. *ganpiniana* (Lévl. et Vant.) W. T. Wang ($2n = 2x = 16$), *C. brevicaudata* DC. ($2n = 2x = 16$), *C. chrysocoma* Franch. ($2n = 2x = 16$). The species of $x = 8$ in *Anemone*, and those in *Anemoclema* and *Clematis* have very similar karyotypes which consist of five pairs of large median-centromeric (rarely one pair of which are submedian-centromeric) and three pairs of subterminal- or terminal-centromeric chromosomes.

Key words Ranunculaceae; *Anemone* L., *Anemoclema* (Franch.) W. T. Wang; *Clematis* L.; Chromosome number; Karyotype

1 Introduction

Anemone L., with about 150 species in the world, and *Clematis* L., with about 240 species, are the two largest genera in the tribe Anemoneae of the Ranunculaceae (Tamura, 1995). These two genera are very richly represented in China, where about 60 species in *Anemone* and more than 100 species in *Clematis* L. have been recorded (Wang, 1979).

Cytological studies in *Anemone* and *Clematis* have been reported in various countries (for *Anemone*: Moffett, 1932; Kurita, 1957a; Heimburger, 1959; Boraiah & Heimburger, 1964; Baumberger, 1970; Bhattarai, 1989; and for *Clematis*: Meurman & Therman, 1939; Kurita, 1957b, 1962; Pringle, 1971; Dennis, 1976; Dawson, 1993). The Chinese species in these two genera, particularly in *Anemone*, however, are cytologically very poorly known. Only 18 species in *Clematis* and one in *Anemone* from China have had reports of chromosome counts and chromosome morphology (Gong *et al.*, 1985; Zhang & He, 1990; Zhang, 1991; Yang *et al.*, 1994).

Molecular data have shown that there are two large clades within *Anemone*, clade Ⅰ with the basic chromosome number $x = 8$, and clade Ⅱ with the basic chromosome number $x = 7$ (Hoot *et*

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al., 1994; Ehrendorfer & Samuel, 2001). This indicates that the chromosome number is of important significance in determining phylogenetic relationships of the species in *Anemone*. This paper is to report the chromosome number and morphology of ten species in *Anemone*, one in *Anemoclema* and six in *Clematis*.

2 Material and Methods

All the plants studied were collected in the field from Yunnan, Sichuan, Shanxi and Qinghai of China (Table 1). They were cultivated in pots in the experimental garden of Kunming Institute of Botany, the Chinese Academy of Sciences, before their root tips were harvested for the cytological study. Actively growing root tips were pretreated in 0.1% colchicine at about 20℃ for 3 hr before they were fixed in Carnoy I (glacial acetic acid : absolute ethanol = 1 : 3) at 4℃ for 30 min, then they were macerated in 1 : 1 mixture of 1 mol/L hydrochloric acid and 45% acetic acid at 60℃ for 2 min, and stained and squashed in 1% aceto-orcein.

Karyotype formulas were based on the measurements of somatic chromosomes. The symbols used to describe the karyotypes followed Levan *et al*. (1964): m = median-centromeric chromosome, sm = submedian-centromeric chromosome, st = subterminal-centromeric chromosome, and t = terminal-centromeric chromosome.

The voucher specimens were deposited in the Herbarium of Institute of Botany, the Chinese Academy of Sciences (PE). Taxonomic treatments and systematic arrangements of the species followed Wang (1979, 2000).

3 Results

3.1 *Anemone* L.

The chromosome counts obtained are given for 11 taxa in Table 1. Of these, two are in each of sections *Anemonanthea* and *Anemone*, one in each of sections *Stolonifera*, *Rivularidum*, *Begoniifolia*, *Omalocarpus*, and three in section *Himalayicae*. Some of these are illustrated in Figures 1 & 2 and Figures 5 ~ 15 and show the chromosome morphology that is typical of the genus, with five large pairs of median-centromeric (rarely one pair of which are submedian-centromeric), and three pairs of subterminal- or terminal-centromeric chromosomes.

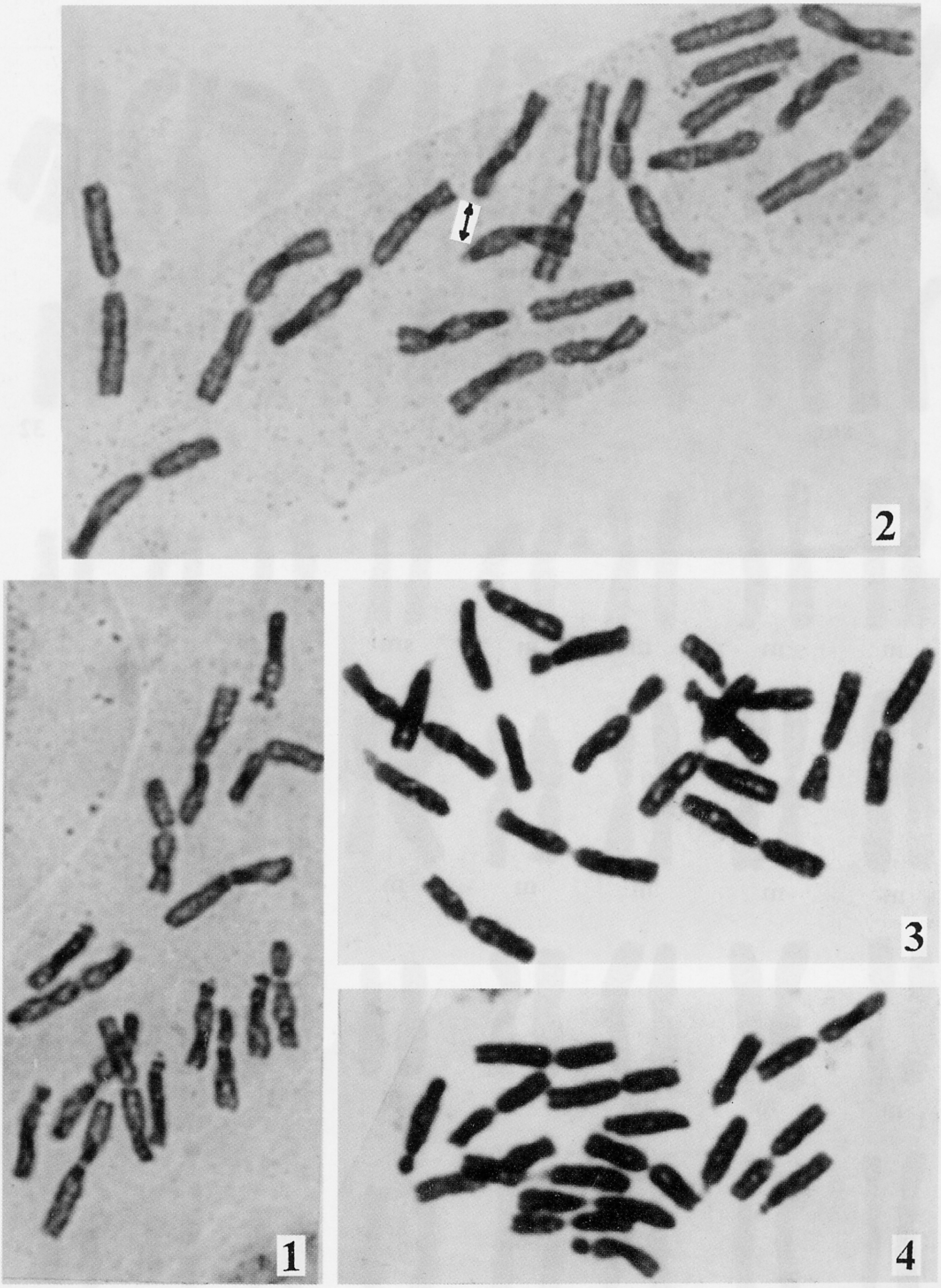
Of the eleven taxa studied, one species, *A*. *davidii*, is tetraploid with 2n = 32 based on x = 8, five species, *A*. *stolonifera*, *A*. *rivularis*, *A*. *begoniifolia*, *A*. *hupehensis* f. *alba*, *A*. *tomentosa*, are diploid with 2n = 16 based on x = 8, and five taxa, *A*. *flaccida*, *A*. *rupestris*, *A*. *trullifolia* var. *colestina*, *A*. *trullifolia* var. *holophylla* and *A*. *demissa* are diploid with 2n = 14 based on x = 7. The chromosome counts in *A*. *davidii*, *A*. *begoniifolia*, *A*. *rupestris*, *A*. *trullifolia* var. *colestina* and *A*. *trullifolia* var. *holophylla* represent new records. Those in the remaining species confirm the reports by previous workers (Kurita, 1957a; Heimburger, 1959; Baumberger, 1970; Bhattarai, 1989).

The chromosomes of *A*. *davidii* can be arranged into 8 groups of 4 homologues (Fig. 5). It is noteworthy that the chromosomes of the fifth group are submedian-centromeric with the arm ratio up to 2.6. The chromosome number of this species from Xinning, Hunan, was counted to be 2n = 16 (Luo Yan, personal communication), indicating ploidy variation in this species.

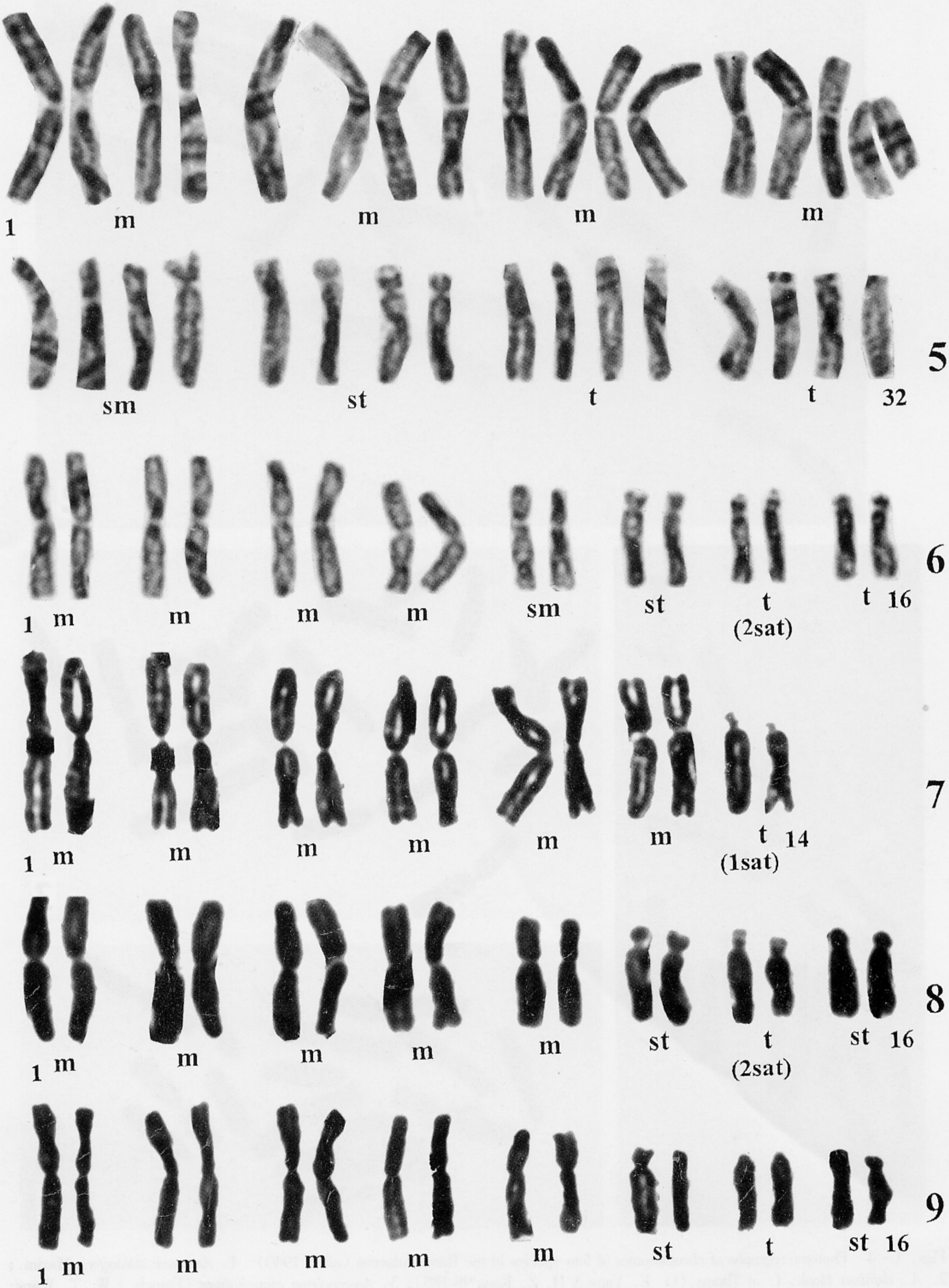
The five species with 2n = 16 (Fig. 6, Figs. 8 ~ 11) have very similar karyotypes, which consist of five pairs of large median-centromeric (in *Anemone stolonifera*, the fifth pair being submedi-

Table 1 Chromosome counts and source of material studied

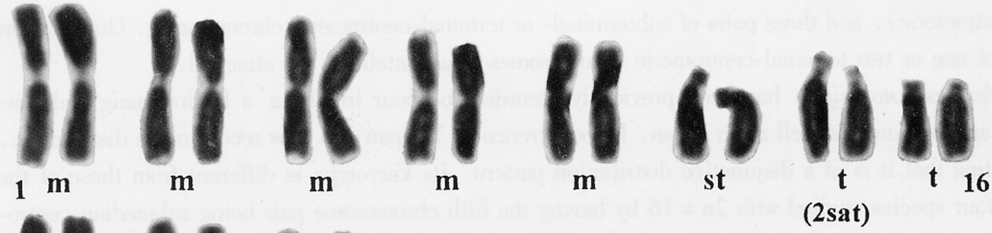
Taxon	Provenance	Voucher	2n
Anemone L.			
Sect. *Anemonanthea* DC.			
A. davidii Franch.	Weixi, Yunnan	Q. E. Yang & H. Z. Kong 98-450	32
A. stolonifera Maxim.	Zhongdian, Yunnan	Q. E. Yang & H. Z. Kong 98-110	16
Sect. *Stolonifera* (Ulbr.) Juz.			
A. flaccida Fr. Schmidt	Dali, Yunnan	Q. E. Yang 94-13	14
	Weixi, Yunnan	Q. E. Yang 94-73	14
Sect. *Rivularidium* Jancz.			
A. rivularis Buch.-Ham.	Lijiang, Yunnan	X. Gong 92-14	16
	Dali, Yunnan	Q. E. Yang 94-23	16
	Zhongdian, Yunnan	X. Gong 92-57	16
	Dawu, Sichuan	Y. B. Luo 88	16
Sect. *Begoniifolia* (Ulbr.) Tamura			
A. begoniifolia Lévl. et Vant.	Nanchuan, Sichuan	Q. E. Yang & M. Feng 95-12	16
Sect. *Anemone*			
A. hupehensis Lem. f. *alba* W. T. Wang	Zhongdian, Yunnan	X. Gong 92-42	16
	Dali, Yunnan	Q. E. Yang 94-12	16
A. tomentosa (Maxim.) Péi	Xunhua, Qinghai	Q. E. Yang 95-05	16
	Jishan, Shanxi	Y. B. Luo 20	16
Sect. *Himalayicae* (Ulbr.) Juz.			
A. rupestris Hook. f. et Thoms.	Zhongdian, Yunnan	Q. E. Yang 94-105	14
A. trullifolia Hook. f. et Thoms. var. *colestina* (Franch.) Finet et Gagnep.	Dali, Yunnan	Q. E. Yang 94-09	14
	Weixi, Yunnan	Q. E. Yang 94-68	14
var. *holophylla* Diels	Zhongdian, Yunnan	Q. E. Yang & H. Z. Kong 98-528	14
Sect. *Omalocarpus* DC.			
A. demissa Hook. f. et Thoms.	Zhongdian, Yunnan	X. Gong 92-41	14
	Weixi, Yunnan	Q. E. Yang & H. Z. Kong 98-185	14
Anemoclema (Franch.) W. T. Wang			
A. glaucifolium (Franch.) W. T. Wang	Lijiang, Yunnan	Q. E. Yang 93-201	16
Clematis L.			
Sect. *Viorna* (Reichb.) Prantl			
Subsect. *Connatae* Koehne			
C. kockiana Schneid.	Yunlong, Yunnan	Q. E. Yang 93-02	16
C. rehderiana Craib.	Zhongdian, Yunnan	Q. E. Yang & H. Z. Kong 98-175	16
	Dêqên, Yunnan	Q. E. Yang & H. Z. Kong 98-405	16
C. ranunculoides Franch.	Yanyuan, Sichuan	Q. E. Yang 93-09	16
	Zhongdian, Yunnan	Q. E. Yang & H. Z. Kong 98-297	16
Subsect. *Vitalbae* Prantl			
C. puberula Hook. f. et Thoms. var. *ganpiniana* (Lévl. et Vant.) W. T. Wang	Lijiang, Yunnan	Q. E. Yang 94-143	16
C. brevicaudata DC.	Dêqên, Yunnan	Q. E. Yang 98-384	16
Sect. *Cheiropsis* DC.			
C. chrysocoma Franch.	Lijiang, Yunnan	Q. E. Yang 94-150	16



Figs. 1 ~ 4 Photomicrographs of chromosomes of four species in the Ranunculaceae (all × 1940) 1. *Anemone stolonifera* Maxim.; 2. *A. demissa* Hook. f. et Thoms. (Q. E. Yang & H. Z. Kong 98-185); 3. *Anemoclema glaucifolium* (Franch.) W. T. Wang; 4. *Clematis puberula* var. *ganpiniana* (Lévl. et Vant.) W. T. Wang


1 m m m m
sm st t t 32
5
1 m m m m sm st t (2sat) t 16
6
1 m m m m m m t (1sat) 14
7
1 m m m m m st t (2sat) st 16
8
1 m m m m m st t st 16
9

Figs. 5~9 Karyotypes in five species of *Anemone* L. (all ×1940) 5. *A. davidii* Franch.; 6. *A. stolonifera* Maxim.; 7. *A. flaccida* Fr. Schmidt (Q. E. Yang 94-13); 8. *A. rivularis* Buch.-Ham. (X. Gong 92-57); 9. *A. begoniifolia* Lévl. et Vant.



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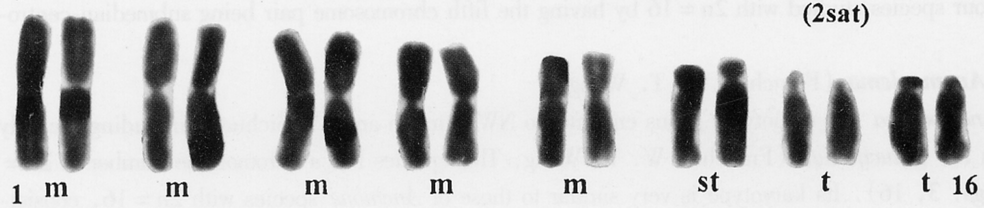

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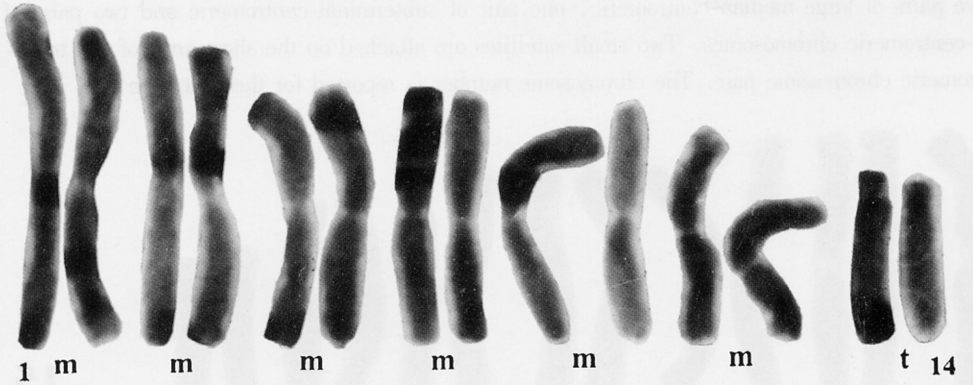

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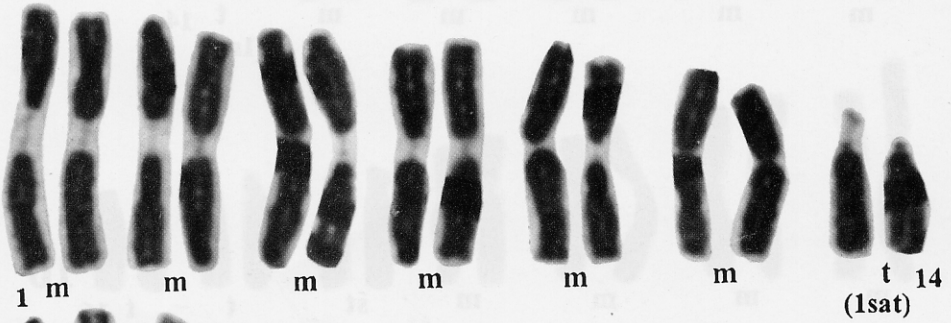

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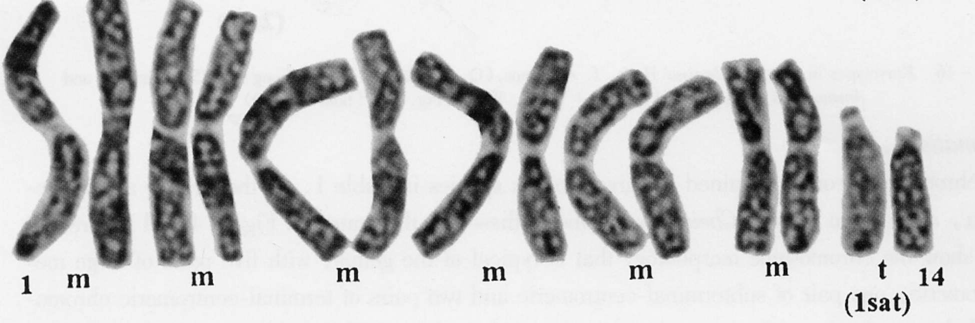

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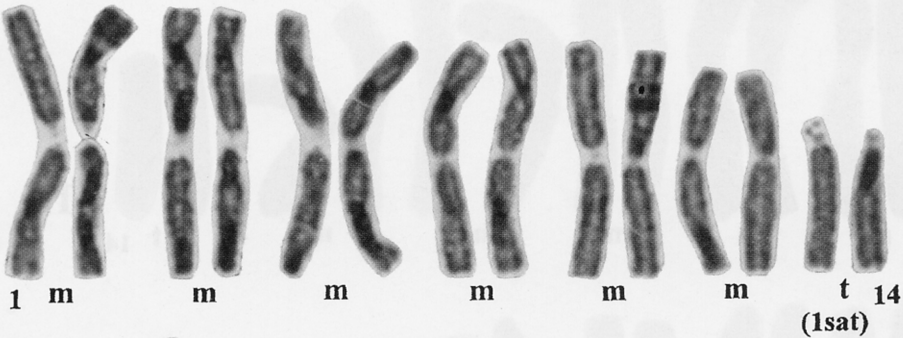
Figs. 10 ~ 14 Karyotypes in five taxa of *Anemone* L. (all × 1940) 10. *A. hupehensis* Lem. f. *alba* W. T. Wang(X. Gong 92-42); 11. *A. tomentosa* (Maxim.) Péi(Q. E. Yang 95-05); 12. *A. rupestris* Hook. f. et Thoms.; 13. *A. trullifolia* var. *colestina* (Franch.) Finet et Gagnep. (Q. E. Yang 94-09); 14. *A. trullifolia* var. *holophylla* Diels.

an-centromeric), and three pairs of subterminal- or terminal-centromeric chromosomes. On the short arms of one or two terminal-centromeric chromosomes small satellites are attached.

Anemone stolonifera has been previously recorded to occur in China's Heilongjiang and Taiwan, and in Korea as well as in Japan. Its occurrence in Yunnan is a new record of its distribution, indicating that it is of a disjunctive distribution pattern. Its karyotype is different from those of the other four species studied with 2n = 16 by having the fifth chromosome pair being submedian-centromeric.

3.2 *Anemoclema* (Franch.) W. T. Wang

Anemoclema is a monotypic genus endemic to NW Yunnan and SW Sichuan, including the only species *A. glaucifolium* (Franch.) W. T. Wang. This species has a chromosome number of 2n = 16 (Figs. 3, 16). Its karyotype is very similar to those of *Anemone* species with 2n = 16, consisting of five pairs of large median-centromeric, one pair of subterminal-centromeric and two pairs of terminal-centromeric chromosomes. Two small satellites are attached on the short arms of one terminal-centromeric chromosome pair. The chromosome number is reported for the first time.



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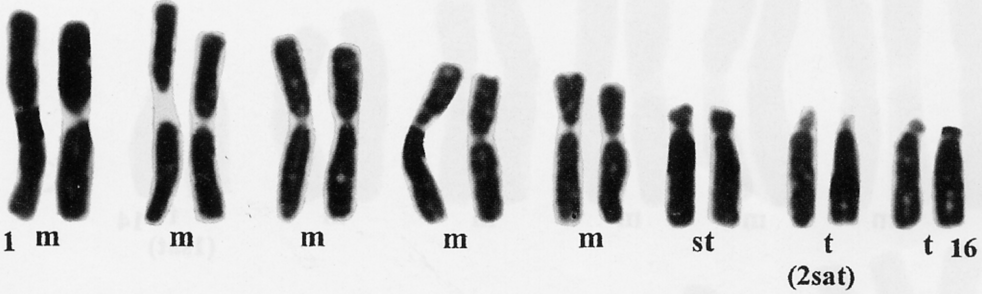

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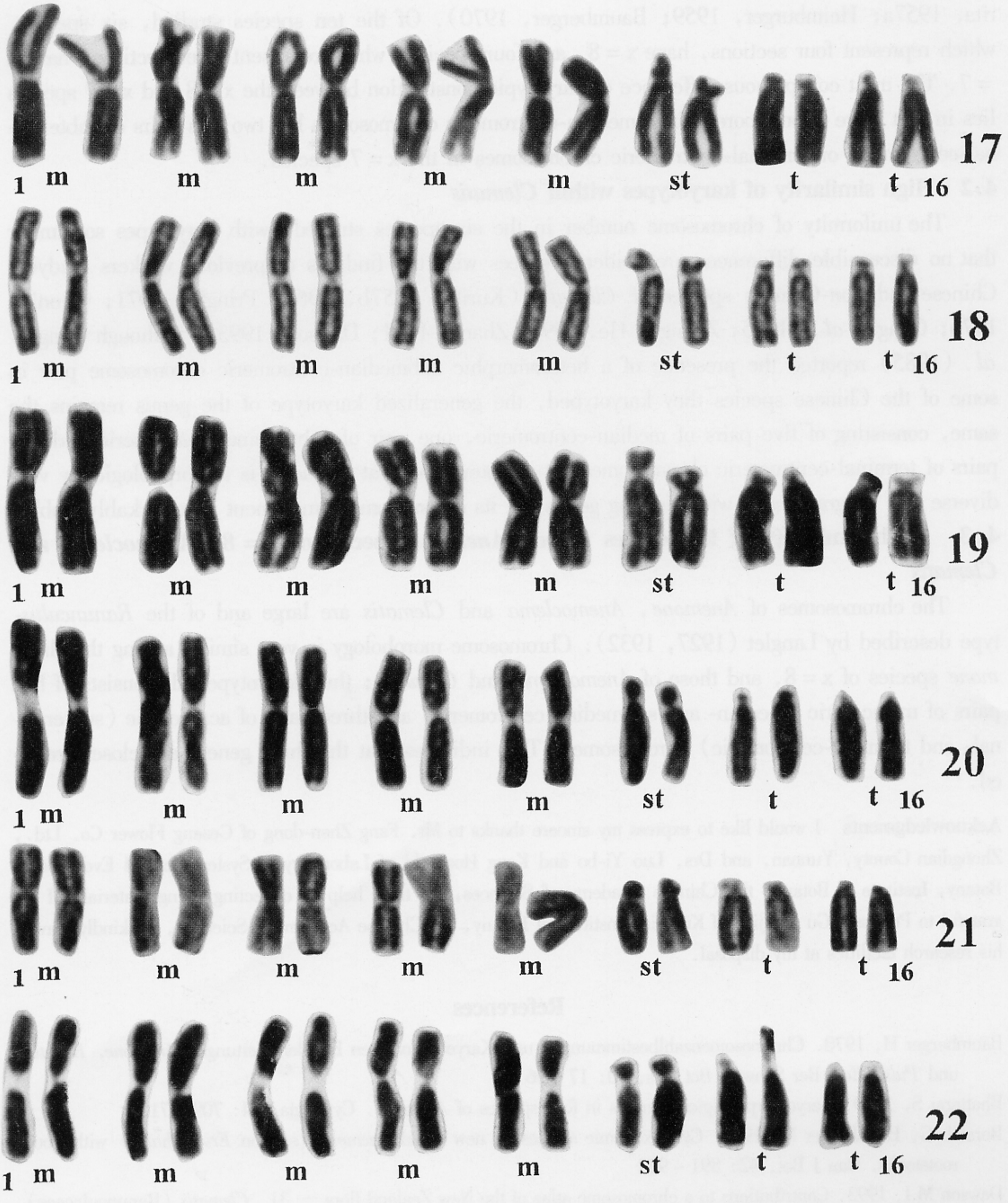
Figs. 15 ~ 16 Karyotypes in *Anemone demissa* Hook. f. et Thoms. (Q. E. Yang & H. Z. Kong 98-185) (Fig. 15) and *Anemoclema glaucifolium* (Franch.) W. T. Wang (Fig. 16) (both × 1940)

3.3 *Clematis* L.

The chromosome counts obtained are given for six species in Table 1. Of these, five are in section *Viorna*, and one in section *Cheiropsis*. Some of these are illustrated in Figure 4 and Figures 17 ~ 22 and show the chromosome morphology that is typical of the genus, with five pairs of large median-centromeric, one pair of subterminal-centromeric and two pairs of terminal-centromeric chromosomes. All the species have the same chromosome number of 2n = 16 based on x = 8. The chromosome counts in *C. kockiana*, *C. rehderiana*, *C. ranunculoides* and *C. chrysocoma* represent new records.



Figs. 17 ~ 22 Karyotypes in six species of *Clematis* L. (all × 1940). 17. *C. kockiana* Schneid.; 18. *C. rehderiana* Craib. (Q. E. Yang & H. Z. Kong 98-175); 19. *C. ranunculoides* Franch. (Q. E. Yang 93-09); 20. *C. puberula* var. *ganpiniana* (Lévl. et Vant.) W. T. Wang; 21. *C. brevicaudata* DC.; 22. *C. chrysocoma* Franch.

4 Discussion

4.1 Basic chromosome numbers in *Anemone* L.

The chromosome counts obtained confirm previous reports that there are two basic chromosome numbers, x = 7 and 8, in the genus *Anemone* (Langlet, 1932; Moffett, 1932; Gregory, 1941; Kurita, 1957a; Heimburger, 1959; Baumberger, 1970). Of the ten species studied, six species, which represent four sections, have x = 8, and four species, which represent three sections, have x = 7. The most conspicuous difference of karyotypic constitution between the x = 8 and x = 7 species lies in that there is one more pair of median-centromeric chromosomes but two less pairs of subterminal-centromeric or terminal-centromeric chromosomes in the x = 7 species.

4.2 High similarity of karyotypes within *Clematis*

The uniformity of chromosome number in the six species studied, with karyotypes so similar that no discernible differences are evident, agrees with the findings of previous workers studying Chinese and non-Chinese species of *Clematis* (Kurita, 1957b, 1962; Pringle, 1971; Dennis, 1976; Gong *et al*., 1985; Zhang & He, 1990; Zhang, 1991; Dawson, 1993). Although Gong *et al*. (1985) reported the presence of a heteromorphic submedian-centromeric chromosome pair in some of the Chinese species they karyotyped, the generalized karyotype of the genus remains the same, consisting of five pairs of median-centromeric, one pair of subterminal-centromeric and two pairs of terminal-centromeric chromosomes. It is noteworthy that *Clematis* is a morphologically very diverse and geographically wide-ranging genus but its chromosome complement is remarkably stable.

4.3 High similarity of karyotypes among *Anemone* species of x = 8, *Anemoclema* and *Clematis*

The chromosomes of *Anemone*, *Anemoclema* and *Clematis* are large and of the *Ranunculus*-type described by Langlet (1927, 1932). Chromosome morphology is very similar among the *Anemone* species of x = 8, and those of *Anemoclema* and *Clematis*; their karyotypes all consists of five pairs of metacentric (median- and submedian-centromeric) and three pairs of acrocentric (subterminal- and terminal-centromeric) chromosomes. This indicates that the three genera are closely related.

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国产毛茛科银莲花族十七种植物的细胞学研究

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摘要　研究了国产毛茛科银莲花族 Trib. Anemoneae 17 种植物的染色体数目和核型。10 种银莲花属 *Anemone* L.植物中,1 种(西南银莲花 *A. davidii*)为 $x = 8$ 的四倍体($2n = 4x = 32$),5 种 (匍枝银莲花 *A. stolonifera*、草玉梅 *A. rivularis*、卵叶银莲花 *A. begoniifolia*、水棉花 *A. hupehensis* f. *alba*、大火草 *A. tomentosa*)为 $x = 8$ 的二倍体 ($2n = 2x = 16$), 4 种 (鹅掌草 *A. flaccida*、湿地银莲花 *A. rupestris*、蓝匙叶银莲花 *A. trullifolia* var. *colestina*、拟条叶银莲花 *A. trullifolia* var. *holophylla*、展毛银莲花 *A. demissa*) 为 $x = 7$ 的二倍体 ($2n = 2x = 14$)。罂粟莲花 *Anemoclema glaucifolium* 为 $x = 8$ 的二倍体。6 种铁线莲属 *Clematis* L. 植物 (滇川铁线莲 *C. kockiana*、长花铁线莲 *C. rehderiana*、毛茛铁线莲 *C. ranunculoides*、扬子铁线莲 *C. puberula* var. *ganpiniana*、短尾铁线莲 *C. brevicaudata*、金毛铁线莲 *A. chrysocoma*)均为 $x = 8$ 的二倍体。银莲花属中 $x = 7$ 的种类的核型彼此十分相似,均由 6 对大型具中部着丝点的染色体和 1 对具端部着丝点的染色体组成;$x = 8$ 的二倍体种类的核型与罂粟莲花属和铁线莲属植物的核型十分相似,均由 5 对大型具中部着丝点和 3 对具端部或近端部着丝点的染色体组成。

关键词　毛茛科; 银莲花属; 罂粟莲花属; 铁线莲属; 染色体数目; 核型